# Quantibody® Rabbit Cytokine Array 1

--Quantitative measurement of 10 Rabbit cytokines

**Patent Pending Technology**

**User Manual (Version June 2014)**

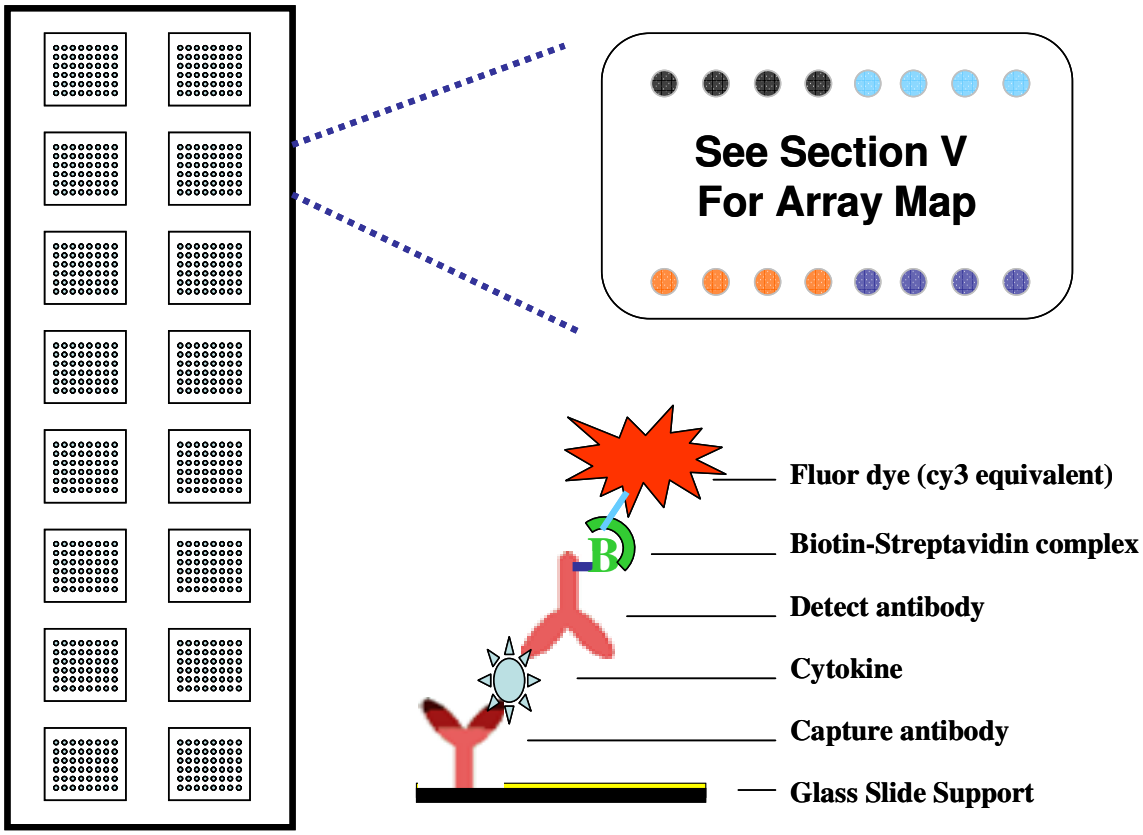
**Cat # QAL-CYT-1**

**RayBiotech, Inc.**

**We Provide You With Excellent**
**Protein Array Systems and Service**

**Tel:(Toll Free) 1-888-494-8555 or 770-729-2992; Fax: 1-888-547-0580;**
**Website:www.raybiotech.com Email: info@raybiotech.com**

| | |
|---|---|
| Cytokine Detected (10) | IL-1alpha, IL-1beta, IL-8, IL-17A, IL-21, Leptin, MIP-1beta, MMP-9, NCAM-1, TNF alpha |
| Format | One standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody is arrayed in quadruplicate. |
| Detection Method | Fluorescence with laser scanner: Cy3 equivalent dye |
| Sample Volume | 50 – 100 µl per array |
| Reproducibility | CV <20% |
| Assay duration | 6 hrs |



# TABLE OF CONTENTS


| | | |
|---|---|---|
| I. | Overview…….…. | 1 |
| | Introduction…..... | 3 |
| | How It Works ……... | 5 |
| II. | Materials Provided…….…….. | 6 |
| | Additional Materials Required……..…… | 6 |
| III. | General Considerations…….…… | 7 |
| | A. Preparation of Samples…….…… | 7 |
| | B. Handling Glass Chips…….…….. | 7 |
| | C. Incubation…….…… | 7 |
| IV. | Protocol…….…. | 8 |
| | A. Complete Air Dry the Glass Chip…… | 8 |
| | B. Prepare Cytokine Standard Dilutions…….. | 8 |
| | C. Blocking and Incubation……. | 9 |
| | D. Incubation with Detection Antibody Cocktail...…. | 10 |
| | E. Incubation with Cy3 Equivalent Dye-Streptavidin … | 10 |
| | F. Fluorescence Detection…… | 11 |
| | G. Data Analysis…….. | 12 |
| V. | Cytokine Array Map & Standard Curves... …… | 13 |
| VI. | 8-Point Standards ……... | 14 |
| VII. | System Recovery …….……... | 15 |
| VIII. | Quantibody® Q-Analyzer ……..….. | 16 |
| IX. | Troubleshooting Guide……..... | 17 |
| X. | Select Quantibody® Publications …… | 18 |
| XI. | Experimental Record Form…… | 19 |
| XII. | How to Choose Quantibody® Products …….. | 20 |

# I. Introduction

Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent array (ELISA). In this method, target protein is first immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme-complex can then be visualized through the use of a substrate that produces a detectable signal. While the traditional method works well for a single protein, the overall procedure is time consuming and requires a lot of sample. With little sample to work with, conservation of precious small quantities becomes a risky task. Take the advantage of advancement in microarray technology over the last decade; more and more choices are available to the scientist today. A long-standing leader in the field, Raybiotech, has pioneered the development of cytokine antibody arrays, which has now been widely applied in the research community with hundreds of peer reviewed publications such as in Cell and Nature.

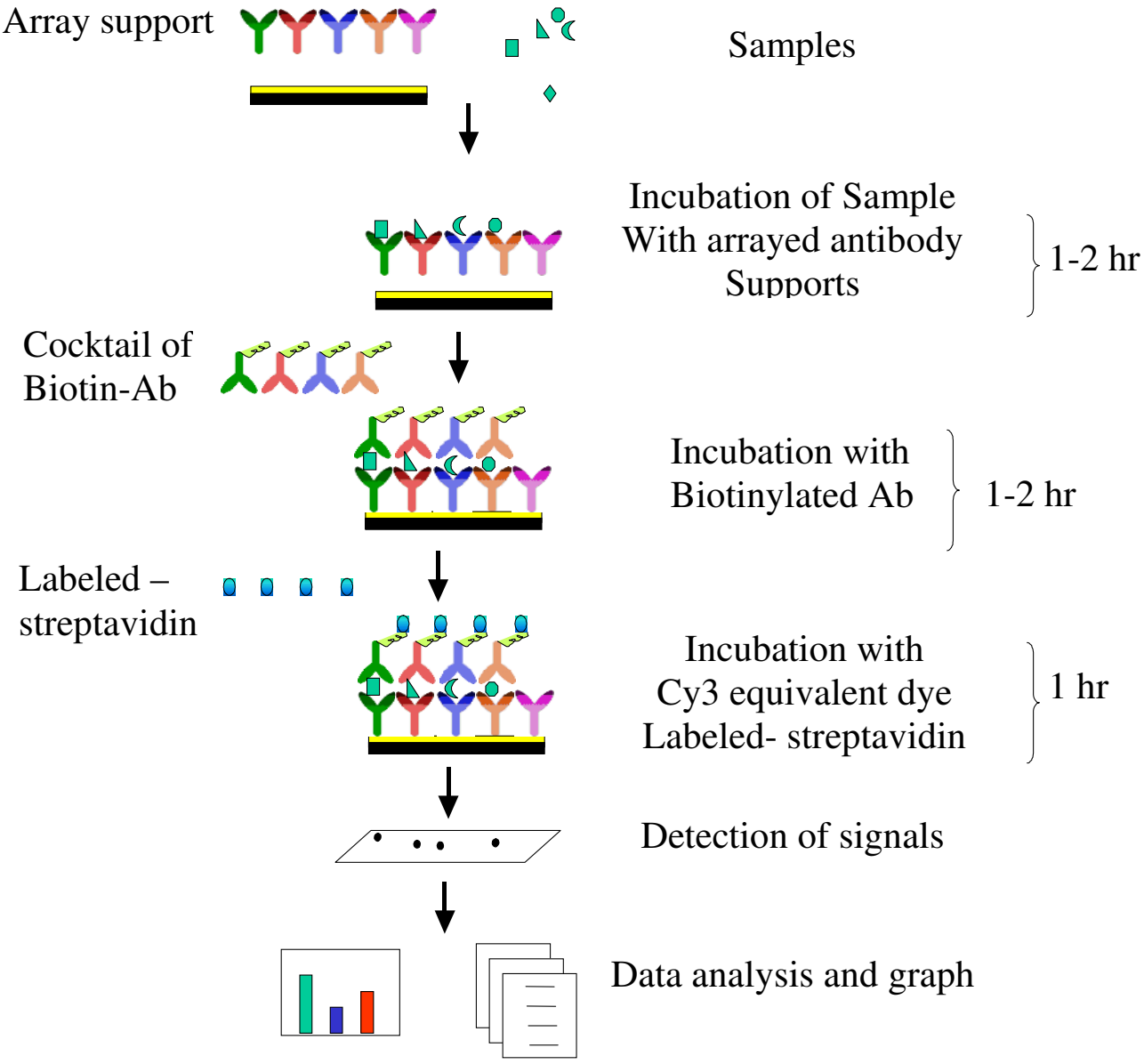
Quantibody® array, our quantitative array platform, uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity / specificity of ELISA and the high throughput of the arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled Cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use array format. By arraying multiple cytokine

specific capture antibodies onto a glass support, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples in one slide. Four slide chips can be nested into a tray, which matches a standard microplate and allows for automated robotic high throughput process of 64 arrays simultaneously. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

Quantibody® array kits have been confirmed to have similar detection sensitivity as traditional ELISA. Our current high density Quantibody kits allow scientists to quantitatively determine the concentration of 400 human or 200 mouse cytokines in a single experiment. This is not only one of the most efficient products on the market for cytokine quantification, but makes it more affordable for quantification of large number of proteins. Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool for drug and biomarker discovery.

# How It Works


Array support
Samples
Incubation of Sample
With arrayed antibody
Supports
1-2 hr
Cocktail of
Biotin-Ab
Incubation with
Biotinylated Ab
1-2 hr
Labeled –
streptavidin
Incubation with
Cy3 equivalent dye
Labeled- streptavidin
1 hr
Detection of signals
Data analysis and graph

# II. Materials Provided

Upon receipt, all components of the Quantibody® Array kit should be stored at -20$^0$C. At -20$^0$C the kit will retain complete activity for up to 6 months. Once thawed, the glass chip, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at –20$^0$C and all other components may be stored at 4$^0$C. The entire kit should be used within 6 months of purchase.

## Components

| Item | Description | 1-Slide kit | 2-Slide kit |
|---|---|---|---|
| 1 | Quantibody® Array Glass Chip | 1 | 2 |
| 2 | Sample Diluent | 1 | 1 |
| 3 | 20X Wash Buffer I | 2 | 3 |
| 4 | 20X Wash Buffer II | 1 | 1 |
| 5 | Lyophilized cytokine standard mix * | 1 | 1 |
| 6 | Detection antibody cocktail | 1 | 2 |
| 7 | Cy3 equivalent dye-conjugated Streptavidin | 1 | 2 |
| 8 | Slide Washer/Dryer | 1 | 1 |
| 9 | Adhesive device sealer | 5 | 10 |
| 10 | Manual | 1 | 1 |

** See Section VI for detailed cytokine concentrations after reconstitution.*

### Additional Materials Required

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- 1.5ml Polypropylene microcentrifuge tubes

# III. General Considerations

## A. Preparation of Samples

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.
- We recommend the following parameters for other samples:
  50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

  *If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.*

## B. Handling glass chips

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Handle glass chip in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, you might not have enough info to distinguish one slide from the other.

## C. Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µl of sample or reagent is used.
- Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at $4^0$C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

# IV. Protocol

## A. Completely air dry the glass chip

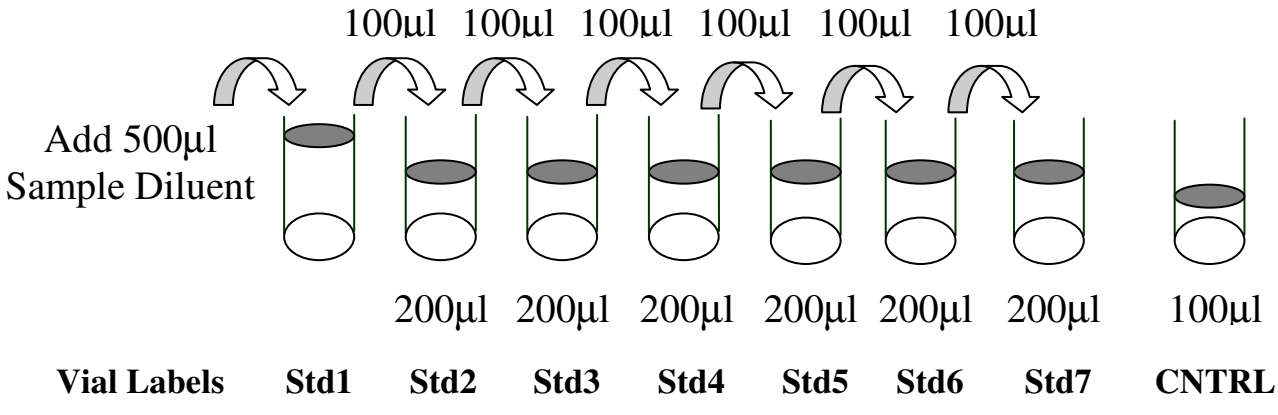
1. Take out the glass chip from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

*Note: Incomplete drying of slides before use may cause the formation of "comet tails".*

## B. Prepare Cytokine Standard Dilutions

*Note: There is only one vial of standard provided in the two-slide kit, which is enough for making two standard curves. Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80 $^{0}$C.*

Prepare serial dilution of cytokine standards


100µl 100µl 100µl 100µl 100µl 100µl
Add 500µl
Sample Diluent
200µl 200µl 200µl 200µl 200µl 200µl 100µl
Vial Labels Std1 Std2 Std3 Std4 Std5 Std6 Std7 CNTRL

2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500µl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1.

3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200µl Sample Diluent to each of the tubes.

4. Pipette 100µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100ul Std2 to tube Std3 and so on.

5. Add 100µl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

*Note: Since the starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in section VI.*

## C. Blocking and Incubation

6. Add 100µl Sample Diluent into each well and incubate at room temperature for 30 min to block slides.

7. Decant buffer from each well. Add 100µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour. *(Longer incubation time is preferable for higher signals)*

   *Note: We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 ul of sample or reagent is used.*

   *Note: This step may be done overnight at $4^0$C for best results.*

8. Wash:
   - Decant the samples from each well, and wash 5 times (5 min each) with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with $H_2O$.

- (*Optional for Cell and Tissue Lysates*) Put the glass chip with frame into a box with 1x Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.

- Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer II with $H_2O$.

*Note: Incomplete removal of the wash buffer in each wash step may cause "dark spots". (Background signal is higher than that of the spot.)*

## D. Incubation with detection antibody cocktail and wash.

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.

10. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour. *(Longer incubation time is preferable for higher signals and backgrounds)*

11. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I and then 2 times with 150 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

## E. Incubation with Cy3 equivalent dye -Streptavidin and wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

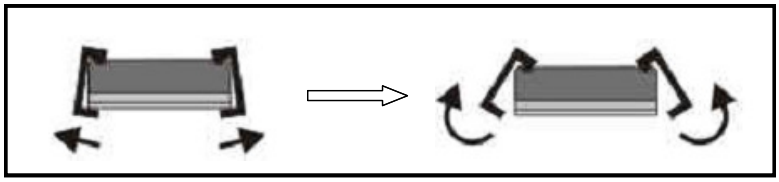
13. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

14. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

## F. Fluorescence Detection

*15.* Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.

*(Be careful not to touch the surface of the array side)*



16. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) with gentle, and gently shake at room temperature for 5 minutes.

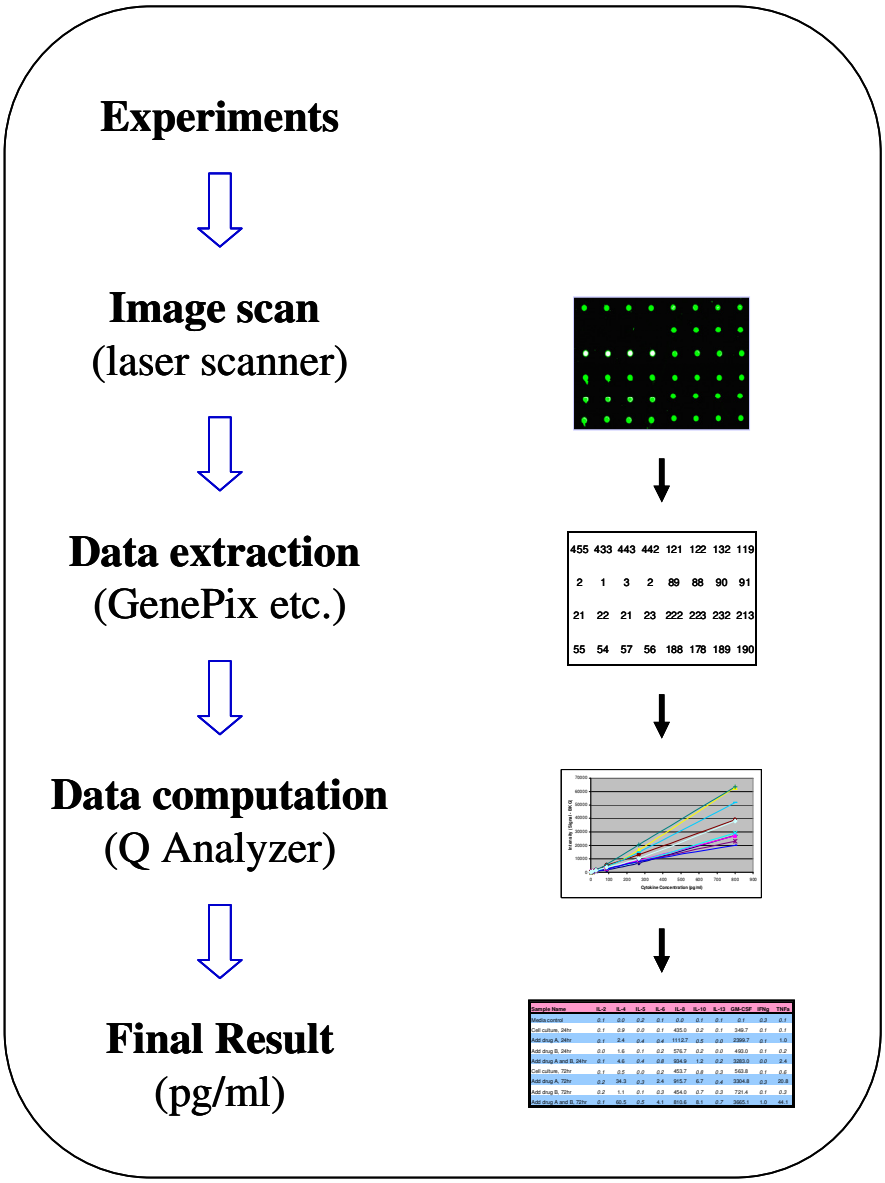
17. Remove water droplets completely by one of the following ways:
    - Dry the glass chip by a compressed $N_2$ stream.
    - Or gently apply suction with a pipette to remove water droplets. Do not touch the array, only the sides.

18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength such as Axon GenePix. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.

*Note: In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.*

## G. Data Analysis

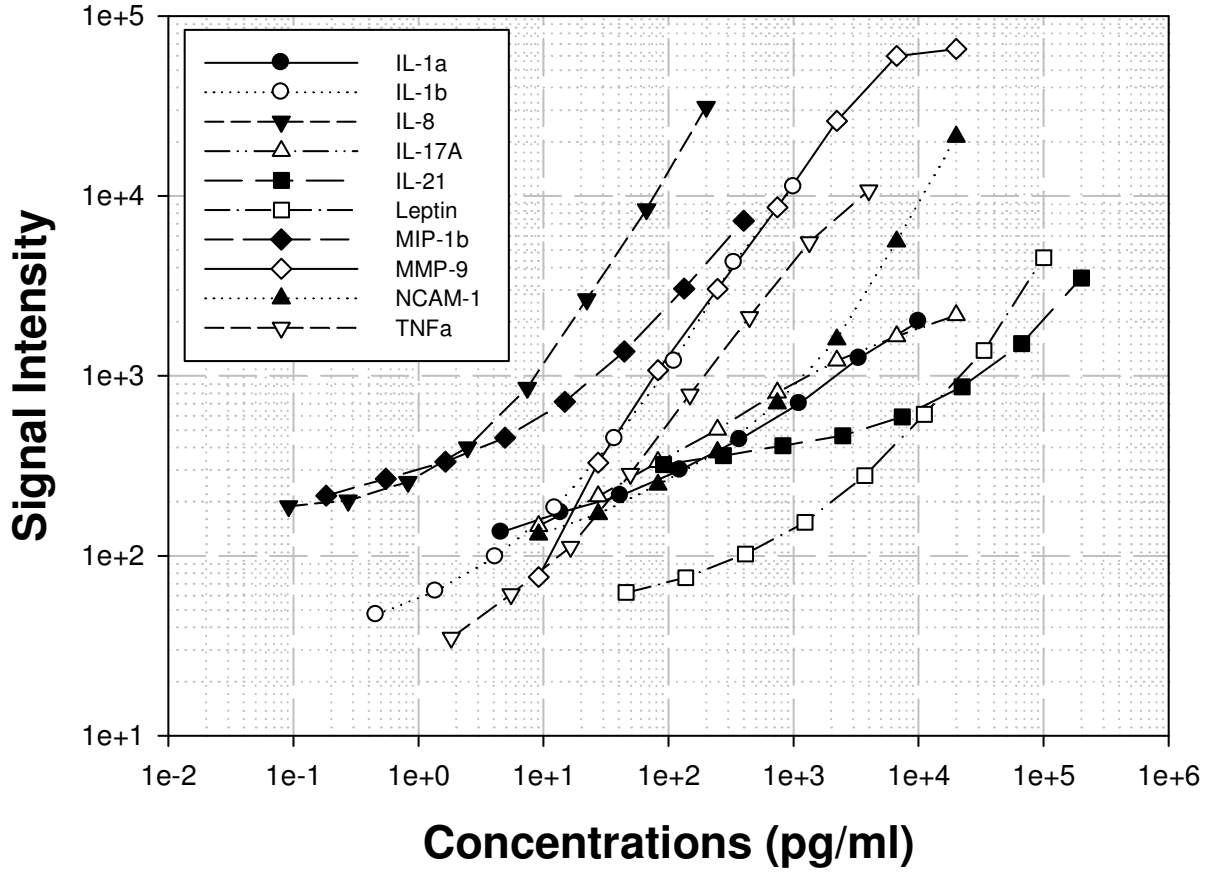
19. Data extraction can be done with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene). For quantitative data analysis, our Quantibody® Q-Analyzer software is available. It gives visual output as well as digital values. More information can be found in section VIII.



# V. Cytokine Array Map & Standard Curves

| POS1 | POS2 |
|---|---|
| IL-1 alpha | IL-1 beta |
| IL-8 | IL-17A |
| IL-21 | Leptin |
| MIP-1 beta | MMP-9 |
| NCAM-1 | TNF alpha |

## QAL-CYT-1 Standard Curves



# VI. 8-Point Standards

After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen is listed below. The detection sensitivity of each protein in one experiment is user dependent. Try our array specific Quantibody Q-Analyzer to see your Limit of Detection (LOD). (Section VIII).

*Serial standard concentration (pg/ml)*

| (pg/ml) | Cntrl | Std7 | Std6 | Std5 | Std4 | Std3 | Std2 | Std1 |
|---|---|---|---|---|---|---|---|---|
| IL-1alpha | 0 | 13.7 | 41.2 | 123.5 | 370.4 | 1,111.1 | 3,333.3 | 10,000 |
| IL-1beta | 0 | 1.4 | 4.1 | 12.3 | 37.0 | 111.1 | 333.3 | 1,000 |
| IL-8 | 0 | 0.3 | 0.8 | 2.5 | 7.4 | 22.2 | 66.7 | 200 |
| IL-17A | 0 | 27.4 | 82.3 | 246.9 | 740.7 | 2,222.2 | 6,666.7 | 20,000 |
| IL-21 | 0 | 274.3 | 823.0 | 2,469.1 | 7,407.4 | 22,222.2 | 66,666.7 | 200,000 |
| Leptin | 0 | 137.2 | 411.5 | 1,234.6 | 3,703.7 | 11,111.1 | 33,333.3 | 100,000 |
| MIP-1beta | 0 | 0.5 | 1.6 | 4.9 | 14.8 | 44.4 | 133.3 | 400 |
| MMP-9 | 0 | 27.4 | 82.3 | 246.9 | 740.7 | 2,222.2 | 6,666.7 | 20,000 |
| NCAM-1 | 0 | 27.4 | 82.3 | 246.9 | 740.7 | 2,222.2 | 6,666.7 | 20,000 |
| TNFalpha | 0 | 5.5 | 16.5 | 49.4 | 148.1 | 444.4 | 1,333.3 | 4,000 |

## VII. System Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. Analysis of samples containing only a single recombinant protein found negligible cross-reactivity with other proteins. The spiking recovery rate of the cytokines by the kit in 2x diluted Rabbit serum and cell culture media is listed in the following table.

*Rabbit Cytokine Array 1 Cross-reactivity Test*

| CAB\DAB | IL-1alpha | IL-1beta | IL-8 | IL-17A | IL-21 | Leptin | MIP-1beta | MMP-9 | NCAM-1 | TNF alpha |
|---|---|---|---|---|---|---|---|---|---|---|
| IL-1alpha | 3,888 | 47 | 21 | 57 | 22 | 46 | 46 | 53 | 58 | 30 |
| IL-1beta | 63 | 20,546 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| IL-8 | 25 | 12 | 31,440 | 33 | 20 | 49 | 28 | 53 | 99 | 20 |
| IL-17A | 76 | 26 | 38 | 3,424 | 49 | 52 | 87 | 66 | 71 | 62 |
| IL-21 | 93 | 127 | 99 | 210 | 2,772 | 116 | 147 | 114 | 163 | 106 |
| Leptin | 5 | 1 | 7 | 36 | 3 | 4,584 | 16 | 9 | 28 | 1 |
| MIP-1beta | 70 | 75 | 69 | 116 | 102 | 137 | 9,934 | 130 | 127 | 89 |
| MMP-9 | 1 | -14 | -7 | 2 | -7 | 3 | -10 | 47,919 | 4 | -4 |
| NCAM-1 | 46 | 29 | 14 | 27 | 37 | 19 | 19 | 16 | 10,063 | 38 |
| TNFalpha | 2 | 1 | 1 | 17 | 12 | 3 | 1 | 2 | 37 | 6,142 |

*The spiking recovery rate for Rabbit serum and culture media (CM)*

| (pg/ml) | Spike-in | CM | CM+Ag | CM% | Serum | Serum+Ag | Serum% |
|---|---|---|---|---|---|---|---|
| IL-1alpha | 5000.0 | 5.1 | 3394.0 | 68% | 22.7 | 3233.7 | 64% |
| IL-1beta | 500.0 | 0.0 | 264.5 | 53% | 0.0 | 467.3 | 93% |
| IL-8 | 100.0 | 0.3 | 109.4 | 109% | 279.5 | 309.4 | 30% |
| IL-17A | 10000.0 | 0.0 | 6280.7 | 63% | 0.0 | 8891.6 | 89% |
| IL-21 | 200000.0 | 0.0 | 206523.0 | 103% | 5539.4 | 180660.5 | 88% |
| Leptin | 50000.0 | 0.0 | 42525.0 | 85% | 419.3 | 62977.8 | 125% |
| MIP-1beta | 200.0 | 2.1 | 252.7 | 125% | 11.0 | 153.4 | 71% |
| MMP-9 | 5000.0 | 0.0 | 2432.7 | 49% | 1188.9 | 6801.0 | 112% |
| NCAM-1 | 10000.0 | 0.0 | 3808.2 | 38% | 64948.3 | 77658.1 | 127% |
| TNFalpha | 2000.0 | 0.0 | 1354.7 | 68% | 6.4 | 2338.1 | 117% |

# VIII. Quantibody® Q-Analyzer

Quantibody Q-Analyzer is an array specific, Excel-based program. However, it is not a simple calculation macro as it contains sophisticated data analysis.

**Key features:**

- *Simplicity:* Easy to operate and requires no professional training. With a simple copy and paste process, the cytokine concentration is determined.
- *Outlier Marking & Removing:* The software can automatically mark and remove the outlier spots for more accurate data analysis
- *Normalization:* The program allows for intra- and inter-slide normalization for large number of samples.
- *Two Positive Controls*: The program takes the two positive controls in each array for normalization.
- *Two Analytical Algorithms*: Users can choose either linear regression or log-log algorithms to meet their analytical needs.
- *Two Data Outputs*: standard curves and digital concentration.
- *User Intervention:* The program allows for user manual handling of those outliers and other analytical data.
- *Lower and Upper Limits Determination:* The program automatically marks out the values below or above the detection range.
- *Standard Deviation:* The program outputs the standard deviations of the quadruplicate spots for data accuracy.
- *Analytical Tips:* Q-Analyzer analysis tips are included in the program.

# IX. Troubleshooting guide

| Problem | Cause | Recommendation |
|---|---|---|
| **Weak Signal** | Inadequate detection | Increase laser power and PMT parameters |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Short incubation time | Ensure sufficient incubation time and change sample incubation step to overnight |
| | Too low protein concentration in sample | Don't make too low dilution or concentrate sample |
| | Improper storage of kit | Store kit as suggested temperature. Don't freeze/thaw the slide. |
| **Uneven signal** | Bubble formed during incubation | Avoid bubble formation during incubation |
| | Arrays are not completed covered by reagent | Completely cover arrays with solution |
| | Reagent evaporation | Cover the incubation chamber with adhesive film during incubation |
| **Poor standard curve** | Cross-contamination from neighboring wells | Avoid overflowing wash buffer |
| | Comet tail formation | Air dry the slide for at least 1 hour before usage |
| | Inadequate standard reconstitution or Improper dilution | Reconstitute the lyophilized standard well at the room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions. |
| | Inadequate detection | Increase laser power that the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated. |
| | Use freeze-thawed cytokine standards | Always use new cytokine standard vial for new set of experiment. Discard any leftover. |
| **High background** | Overexposure | Lower the laser power |
| | Dark spots | Completely remove wash buffer in each wash step. |
| | Insufficient wash | Increase wash time and use more wash buffer |
| | Dust | Work in clean environment |
| | Slide is allowed to dry out | Don't dry out slides during experiment. |

# X. Select Quantibody Publications


1. Stechova, et al. Influence of Maternal Hyperglycaemia on Cord Blood Mononuclear Cells in Response to Diabetes-associated Autoantigens. *Scandinavian Journal of Immunology*. 2009. 70(2):149-158
2. Willingham, SB et al. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. *J Immunol.* 2009; 183(3):2008-15
3. El Karim et al. Neuropeptides Regulate Expression of Angiogenic Growth Factors in Human Dental Pulp Fibroblasts. *Journal of Endodontics*, 2009; 35(6): 829-833
4. Souquière S. et al. T-Cell tropism of simian T-cell leukaemia virus type 1 and cytokine profiles in relation to proviral load and immunological changes during chronic infection of naturally infected mandrills (*Mandrillus sphinx*). *J Med Primatol*. 2009; 38(4):279-89
5. Sharma, et al. Induction of multiple pro-inflammatory cytokines by respiratory viruses and reversal by standardized *Echinacea*, a potent antiviral herbal extract. *Antiviral Research*. 2009; 83(2)165-170.
6. Altamirano-Dimas, et al. *Echinacea* and anti-inflammatory cytokine responses: Results of a gene and protein array analysis. *Pharmacuetical Biology*. 2009; 47(6): 500-508.
7. Cheung, et al. Cordysinocan, a polysaccharide isolated from cultured *Cordyceps*, activates immune responses in cultured T-lymphocytes and macrophages: Signaling cascade and induction of cytokines. *Journal of Ethonopharmacology*. 2009; 124(1): 61-68.
8. Du, et al. P2-380: Identification and characterization of human autoantibodies that may be used for the treatment of prion diseases. *Alzheimer's and Dementia*. 2009; 4(4): T484-T484.
9. Van Rossum et al. Granulocytosis and thrombocytosis in renal cell carcinoma: a pro-inflammatory cytokine response originating in the tumour. *Neth J Med*. 2009; 67(5):191-4.
10. Zhai, et al. Coordinated Changes in mRNA Turnover, Translation, and RNA Processing Bodies in Bronchial Epithelial Cells following Inflammatory Stimulation. *Molecular and Cellular Biology*. 2008; 28(24): 7414-7426.
11. Gao, et al. A Chinese herbal decoction, Danggui Buxue Tang, activates extracellular signal-regulated kinase in cultured T-lymphocytes. *FEBS Letters*, 2007; 581(26): 5087-5093. (This reference validates mulitplex ELISA results for several analytes with standard ELISA test results).
12. Piganelli, et al: Autoreactive T-cell responses: new technology in pursuit of an old nemesis. (Editorial Review) *Pediatric Diabetes* 2007: 8: 249–251

# XI. Experiment Record Form

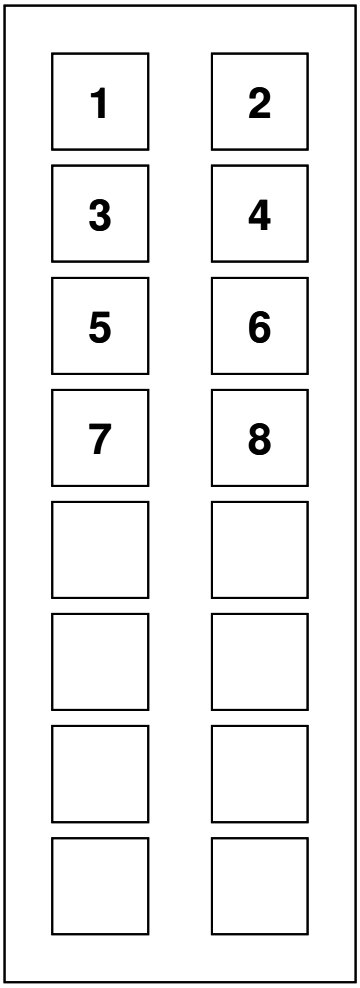
Date: _____

File Name: _____

Laser Power: _____

PMT: _____

| Well No. | Sample Name | Dilution factor |
|---|---|---|
| 1 | CNTRL | |
| 2 | Std7 | |
| 3 | Std6 | |
| 4 | Std5 | |
| 5 | Std4 | |
| 6 | Std3 | |
| 7 | Std2 | |
| 8 | Std1 | |
| 9 | | |
| 10 | | |
| 11 | | |
| 12 | | |
| 13 | | |
| 14 | | |
| 15 | | |
| 16 | | |



# XII. How to Choose Quantibody® Products?

## Species-based selection:

- **Human**: (QAH-)
- **Mouse**: (QAM-)
- **Rat**: QAR
- **Porcine**: QAP-CYT-1
- **Non-Human Primates (NHP)**: QAN-CYT-1
- **Canine**: QAC-CYT-1
- **Feline**: QAF-CYT-1
- **Equine**: QAE-CYT-1
- **Bovine**: QAB-CYT-1
- **Rabbit**: QAL-CYT-1

## Function-based selection:

- **TH1/TH2/TH17 Array:** QAH-TH-1, QAH-TH17, QAM-TH17
- **Inflammation Arrays:** QAH-INF-1, QAH-INF-2, QAH-INF-3; QAM-INF-1; QAR-INF-1
- **Angiogenesis Arrays:** QAH-ANG-1, QAH-ANG-2, QAH-ANG-3, QAH-ANG-1000
- **Chemokine Arrays:** QAH-CHE-1, QAM-CHE-1
- **MMP Array:** QAH-MMP-1
- **Immunoglobin Isotype Array:** QAH-ISO-1; AAM-ISO-G1
- **Dried Eye Disease**: QAH-DED-1
- **Periodontal Disease**: QAH-PDD-1
- **Sepsis Biomarker**: QAH-SEP-1

## Cytokine Number-based selection:

- **400 cytokines:** QAH-CAA-9000
- **360 cytokines:** QAH-CAA-8000
- **320 cytokines:** QAH-CAA-7000
- **280 cytokines:** QAH-CAA-6000
- **240 cytokines:** QAH-CAA-5000
- **200 cytokines:** QAH-CAA-4000; QAM-CAA-4000
- **160 cytokines:** QAH-CAA-3000, QAM-CAA-3000
- **120 cytokines:** QAH-CAA-2000; QAM-CAA-2000
- **80 cytokines:** QAH-CAA-1000; QAM-CAA-1000
- **60 cytokines:** QAH-ANG-1000; QAM-CYT-Q2000
- **40 cytokines:** QAH-INF-3, QAH-CHE-1, QAH-GF-1, QAH-REC-1, QAH-CYT-4,
- **30 cytokines:** QAH-ANG-2, QAH-ANG-3, QAM-INT-1000, QAR-CYT-3, QAM-CHE-1
- **20 cytokines:** QAH-CYT-1, QAH-CYT-2, QAM-CYT-1, QAM-CYT-2, QAM-CYT-3
- **10 cytokines:** QAH-TH-1, QAH-INF-1, QAH-INF-2, QAH-ANG-1, QAH-MMP-1
- **less than 10 cytokines:** QAH-ISO-1, QAH-ADI-2, QAP-CYT-1, QAM-ISO-G1

## Purpose-based selection --- Custom Arrays

- Choose from over 1000 cytokine pool; Any kind; Any number
- Order slide only or full service in house
- Desired marker not in our pool? No problem! For certain developmental fee, we may be able to add the marker to your panel if the paired antibodies are available on the market.

**Note:**

Quantibody® is the trademark of RayBiotech, Inc.

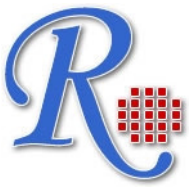
Cytokine protein arrays are RayBiotech patent-pending technology.

This product is intended for research only and is not to be used for clinical diagnosis. Our produces may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for three months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

This product is for research use only.



*©2014 RayBiotech, Inc.*